Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Supplementary Appendix

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Supplemental Methods

Viruses and cells. VeroE6-TMPRSS2 cells were generated by transfecting VERO E6 cells (ATCC CRL-1586) with pCAGGS plasmid in which chicken actin gene promoter drives the expression of an open reading frame comprising Puromycin N-acetyl transferase, GSG linker, 2A self-cleaving peptide of thosea asigna virus (T2A), human transmembrane serine protease 2 (TMPRSS2). Two days post-transfection, cells were trypsinzed and transferred to a 100 mm dish containing complete DMEM medium (1x DMEM, Thermo Fisher, # 11965118, 10% FBS, 1x penicillin/streptomycin) supplemented with puromycin (Thermo Fisher, #A1113803) at a final concentration of 10 µg/ml. Approximately ten days later, individual colonies of cells were isolated using cloning cylinders (Sigma) and expanded in medium containing puromycin. Clonal cell lines were screened for expression of TMPRSS2 by flow cytometry. VeroE6-TMPRSS2 cells were cultured in complete DMEM in the presence of Gibco Puromycin 10mg/mL (# A11138-03). nCoV/USA_WA1/2020 (WA/1), closely resembling the original Wuhan strain and resembles the spike used in the mRNA-1273 and Pfizer BioNTech vaccine, was propagated from an infectious SARS-CoV-2 clone as previously described[1]. icSARS-CoV-2 was passaged once to generate a working stock. hCoV-19/USA/CA-Stanford-15_S02/2021 (herein referred to as the B.1.617.1 variant) was derived from a mid-turbinate nasal swab collected from a Stanford Healthcare patient in March 2021, as part of a study approved by the institutional review board at Stanford University (Protocol 57519). As described previously[2,3], viral genome enrichment was conducted using laboratory-developed, multiplex RT-PCR reactions that generate multiple overlapping amplicons ~1200 base-pairs in length. Fragment libraries were prepared using NEBNext DNA Library Prep reagents for Illumina (New England BioLabs, Ipswich, MA), and were sequenced on an Illumina MiSeq using singleend 150 cycle sequencing using MiSeq Reagent kit V3. The genome was assembled via a custom assembly and bioinformatics pipeline using NCBI NC_045512.2 as reference. 724x mean whole genome coverage was obtained for this sample; 100x coverage was obtained over 93.4% of the genome and 99.9% of spike. The consensus SARS-CoV-2 genome is available under GISAID accession number EPI_ISL_1675223. The B.1.617.1 variant was plaque purified on VeroE6-TMPRSS2 cells and propagated once in a 12-well plate of confluent VeroE6-TMPRSS2 cells followed by expansion of the working stock in a T175 flask of confluent VeroE6-TMPRSS2 cells. The resulting stock was aliquoted to generate a working stock and deep sequenced (**Supplementary Table S1**). Illumina-ready libraries were generated using NEBNext Ultra II RNA Prep reagents (New England BioLabs) as previously described[4]. Briefly, we fragmented RNA, followed by double-stranded cDNA synthesis, end repair, and adapter ligation. The ligated DNA was then barcoded and amplified by a limited cycle PCR and the barcoded Illumina libraries were sequenced by using paired-end 150-base protocol on a NextSeq 2000 (Illumina). Demultiplexed sequence reads were analyzed in the CLC Genomics Workbench v.21.0.3 by (i) trimming for quality, length, and adaptor sequence, (ii) mapping to the Wuhan-Hu-1 SARS-CoV-2 reference (GenBank accession number: NC_045512), (iii) improving the mapping by local realignment in areas containing insertions and deletions (indels), and (iv) generating both a sample consensus sequence and a list of variants. Default settings were used for all tools. hCoV-19/USA/PHC658/2021 (herein referred to as the B.1.617.2 variant) was derived from nasal swab collected in May 2021. The virus was isolated on VeroE6 cells expressing hACE2 and TMPRSS2 and propagated on VeroE6 cells expressing TMPRSS2 (JCRB1819 Japanese Collection of Research Bioresources)[5]. The viral genome was enriched using a multiplex RT-PCR with 345 overlapping amplicons ranging from 75 to 275 nucleotides with an average of 102 base pairs as specified in the SARS-CoV-2 Amplicon Panel (Swift Biosciences, Ann Arbor, MI). The amplicon libraries were purified, size selected, pooled, and normalized using the Swift amplicon panel and Normalase protocols. The sample pool was sequenced on an Illumina MiSeq using paired-end 300 cycle MiSeq Reagent kit v2 (Illumina, San Diego, CA). The sequences were assembled using a custom workflow in CLC Genomics Workbench v21.0.3 (Qiagen, Hilden, Germany) with NCBI NC_045512 as a reference. The genome had a mean coverage of 2692x, with 91.3% of the genome with ≥100x coverage. The spike gene had a mean of 922x coverage and 82.3% had coverage greater than 100x (**Supplementary Table S2**). The B.1.617.2 variant was plaque purified on VeroE6-TMPRSS2 cells and propagated once in a 12-well plate of confluent VeroE6-TMPRSS2 cells followed by expansion of the working stock in a T175 flask of confluent VeroE6-TMPRSS2 cells. The resulting stock was aliquoted to generate a working stock.

Samples. For samples from Emory University, collection and processing were performed under the University Institutional Review Board protocols #00001080 and #00022371. Adults ≥18 years were enrolled who met eligibility criteria for SARS-CoV-2 infection and provided informed consent. Convalescent samples were a convenience sample of individuals that had recovered from mild or moderate COVID-19. Patients had PCR or rapid antigen-test confirmed COVID-19 between the months of March-August 2020 and enrolled with samples collected from May-October 2020[6-8]. These individuals were recruited using multiple methods, including advertisements on the university campus, primary care clinics and at COVID-19 testing sites. Interested participants contacted the clinical research site and underwent a phone screening to assess if they met eligibility criteria. In addition, primary care clinic patients who were being managed for COVID-19 were contacted to see if they are interested in participating in this study.

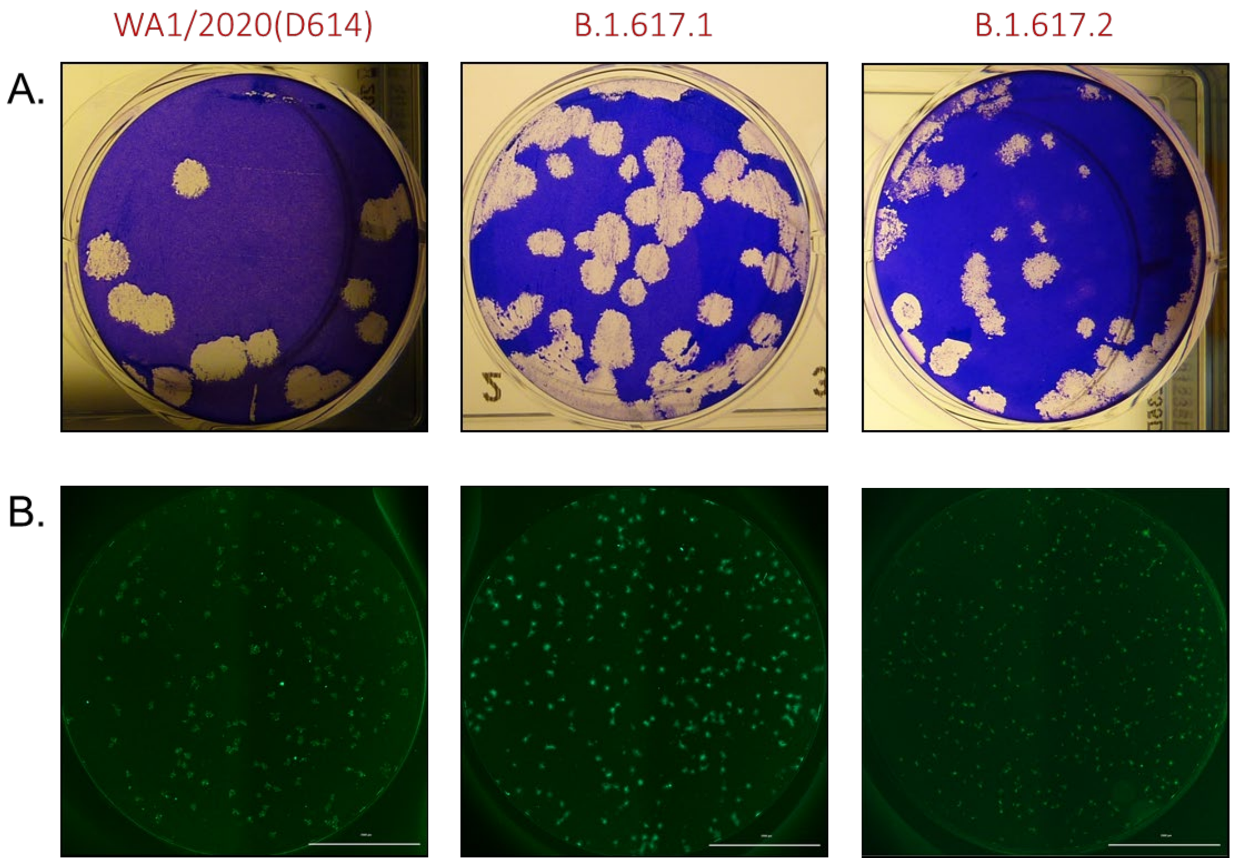
At the time of study enrollment, some of the participants had residual symptoms but others had recovered with no residual symptoms. No data was collected on the number of patients that were pre-screened or declined participation. The Pfizer serum samples were deposited by Florian Krammer, Ph.D. and Viviana Simon, Ph.D., Icahn School of Medicine at Mount Sinai, New York, New York, USA and was obtained through BEI Resources, NIAID, NIH: SARS-CoV-2 Vaccine Human Serum, NR-55279. Plasma was collected from volunteers, previously vaccinated with the Moderna vaccine, at the Emory Hope Clinic and Emory Children's Center under IRB approvals 00045821 and 00022371, respectively. The study enrolled and obtained informed consent from adult participants who had received the complete schedule of the Moderna vaccine. The description of the serum samples from convalescent individuals is shown in **Supplementary Table S3-S4** and from Moderna (mRNA-1273) vaccinated individuals in **Supplementary Table S5** and from Pfizer-BioNTech (BNT162b2) vaccinated individuals in **Supplementary Table S6**.

Focus Reduction Neutralization Test. FRNT assays were performed as previously described[9]. Briefly, samples were diluted at 3-fold in 8 serial dilutions using DMEM (VWR, #45000-304) in duplicates with an initial dilution of 1:10 in a total volume of 60 µl. Serially diluted samples were incubated with an equal volume of WA1/2020 or B.1.617.1 or B.1.617.2 (100-200 foci per well based on the target cell) at 37^{o} C for 1 hour in a round-bottomed 96-well culture plate. The antibody-virus mixture was then added to VeroE6-TMPRSS2 cells and incubated at 37^{o} C for 1 hour. Post-incubation, the antibody-virus mixture was removed and 100 µl of pre-warmed 0.85% methylcellulose (Sigma-Aldrich, #M0512-250G) overlay was added to each well. Plates were incubated at 37^{o} C for 16 hours. After 16 hours, methylcellulose overlay was removed, and cells were washed three times with PBS. Cells were then fixed with 2% paraformaldehyde in PBS for

30 minutes. Following fixation, plates were washed twice with PBS and 100 µl of permeabilization buffer, was added to the fixed cells for 20 minutes. Cells were incubated with an anti-SARS-CoV spike primary antibody directly conjugated with alexaflour-488 (CR3022-AF488) for up to 4 hours at room temperature. Cells were washed three times in PBS and foci were visualized and imaged on an ELISPOT reader (CTL).

Quantification and Statistical Analysis. Antibody neutralization was quantified by counting the number of foci for each sample using the Viridot program[10]. The neutralization titers were calculated as follows: 1 - (ratio of the mean number of foci in the presence of sera and foci at the highest dilution of respective sera sample). Each specimen was tested in duplicate. The FRNT-50 titers were interpolated using a 4-parameter nonlinear regression in GraphPad Prism 8.4.3. Samples that do not neutralize at the limit of detection at 50% are plotted at 15 and was used for geometric mean calculations.

Supplemental Figure S1.



Supplemental Figure S1. Plaque and focus-forming morphology of WA1/2020, B.1.617.1 and B.1.617.2 variants. Monolayers of VeroE6-TMPRSS2 cells were infected with SARS-CoV-2 strains WA1/2020, B.1.617.1 and B.1.617.2. A. Plaque morphology- At 60 hours post-infection, monolayers were fixed with 4% paraformaldehyde for 30 minutes and stained with 0.1% crystal violet in 20% methanol for 10 minutes. were plaqued on a monolayer of VeroE6-TMPRSS2 cells. B. Foci morphology- Focus forming assay was performed as described in the methods. At 16 hours post-infection, monolayers were fixed with 2% paraformaldehyde for 30 minutes, washed with PBS, permeabilized, and stained with a AF488-conjugated CR3022 monoclonal antibody. Cells were visualized using a Cytation7 imager.

Supplemental Table S1. Nucleotide variants and Amino acid mutations identified by deep sequencing results of the B.1.617.1 variant.

Type	Variant	Amino Acid Mutation	Gene (Region)
SNP	3037C>T	--	orf1ab
SNP	3457C>T	--	orf1ab
SNP	4780C>T	--	orf1ab
SNP	4965C>T	T1567I	orf1ab (nsp3)
SNP	5907C>T	T1881I	orf1ab (nsp3)
SNP	11201A>G	T3646A	orf1ab (nsp6)
SNP	14408C>T	P4715L	orf1ab (nsp12)
SNP	16362C>T	--	orf1ab
SNP	17523G>T	M5753I	orf1ab (nsp13)
SNP	18511C>T	--	orf1ab
SNP	20016C>T	--	orf1ab
SNP	20396A>G	K6711R	orf1ab (nsp15)
SNP	20936C>T	T6891M	orf1ab (nsp16)
SNP	21895T>C	--	spike
SNP	21987G>A	G142D	spike
SNP	22022G>A	E154K	spike
SNP	22917T>G	L452R	spike
SNP	23012G>C	E484Q	spike
SNP	23403A>G	D614G	spike
SNP	23604C>G	P681R	spike
SNP	24775A>T	Q1071H	spike
SNP	24863C>G	H1101D	spike
SNP	25276C>T	--	spike
SNP	25469C>T	S26L	orf3a
SNP	26256C>A	F4L	E
SNP	27299T>C	I33T	orf6
SNP	27638T>C	V82A	orf7a
SNP	28881G>T	G202W	N
SNP	29402G>T	--	N

Supplemental Table S2. Nucleotide variants and Amino acid mutations identified by deep sequencing results of the B.1.617.2 variant

Type	Variant	Amino Acid Mutation	Gene (Region)
SNP	926C>T	P309L	orf1ab (nsp2)
SNP	1002C>T	--	orf1ab (nsp2)
SNP	2772C>T	--	orf1ab (nsp3)
SNP	4919C>T	P1640L	orf1ab (nsp3)
SNP	8938G>A	D2980N	orf1ab (nsp4)
SNP	9413T>C	F3138S	orf1ab (nsp4)
SNP	10740C>A	H3580Q	orf1ab (nsp6)
SNP	11970A>C	E3990D	orf1ab (nsp8)
SNP	14144C>T	P4715L	orf1ab (rdrp)
SNP	17232A>G	--	orf1ab (helicase)
SNP	20132A>G	K6711R	orf1ab (endoRNAse)
SNP	56C>G	T19R	S
SNP	230A>C	K77T	S
SNP	425G>A	G142D	S
Deletion	467-472del (AGTTCA)	Del (E156-R158); Ins G	S
SNP	1355T>G	L452R	S
SNP	1433C>A	T478K	S
SNP	1841A>G	D614G	S
SNP	2042C>G	P681R	S
SNP	2848G>A	D950N	S
SNP	77C>T	S26L	orf3a
SNP	245T>C	I82T	M
SNP	359C>T	T120I	orf7a
SNP	305G>T	C102F	orf8
SNP	360C>T	--	orf8
SNP	188A>G	D63G	N
SNP	425C>A	P142Q	N
SNP	608G>T	R203M	N
SNP	1129G>T	D377Y	N
SNP	1239T>C	--	N

Supplemental Table S3. Convalescent COVID-19 samples clinical information

Sample	Age	Sex	Date samples collected	Days After Symptom Onset	Disease Severity
1	65	F	4/17/20	34	Mild
2	48	M	4/22/20	44	Mild
3	51	M	4/22/20	31	Mild
4	29	F	4/22/20	37	Mild
5	36	F	4/23/20	39	Mild
6	37	M	4/23/20	40	Moderate
7	46	M	4/27/20	30	Mild
8	77	F	4/28/20	46	Mild
9	66	M	4/28/20	42	Mild
10	58	M	4/29/20	50	Mild
11	65	M	4/30/20	48	Mild
12	63	F	5/6/20	47	Mild
13	58	F	5/8/20	56	Moderate
14	49	M	5/13/20	61	Mild
15	56.4	M	5/15/20	60	Mild
16	42	F	5/21/20	57	Mild
17	32	F	7/29/20	31	Mild
18	42	M	8/21/20	52	Moderate
19	62	M	8/14/20	83	Severe
20	73	F	8/21/20	50	Severe
21	60	F	8/21/20	76	Severe
22	54	M	8/26/20	58	Moderate
23	28	M	8/26/20	42	Moderate
24	46	M	10/9/20	91	Mild

Supplemental Table S4. FRNT50 results from convalescent COVID-19 samples

	Assay-1			Assay-2		
Sample	**WA1/2020 FRNT50**	**B.1.617.1 FRNT50**	**Fold Change**	**WA1/2020 FRNT50**	**B.1.617.2 FRNT50**	**Fold Change**
1	359	18	19.9	301	66	4.5
2	479	49	9.8	462	127	3.6
3	423	105	4.0	522	152	3.4
4	74	15	4.9	89	15	5.9
5	305	42	7.3	526	103	5.1
6	1129	487	2.3	1109	760	1.5
7	283	15	18.9	297	104	2.9
8	716	100	7.2	600	545	1.1
9	1101	300	3.7	955	603	1.6
10	1088	116	9.4	2179	655	3.3
11	975	72	13.5	1553	366	4.2
12	820	47	17.4	720	426	1.7
13	622	119	5.2	785	135	5.8
14	646	143	4.5	587	305	1.9
15	169	15	11.3	166	73	2.3
16	400	87	4.6	405	237	1.7
17	62	15	4.1	81	83	1.0
18	626	80	7.8	656	208	3.1
19	3085	1296	2.4	1410	1313	1.1
20	944	89	10.6	512	143	3.6
21	425	67	6.3	338	253	1.3
22	423	63	6.7	274	70	3.9
23	819	308	2.7	945	721	1.3
24	734	167	4.4	440	246	1.8

Supplemental Table S5. FRNT50 results from Moderna (mRNA-1273) vaccinated samples

					Assay-1			Assay-2		
Sample	**Age**	**Sex**	**Date samples collected**	**Days After Second Dose**	**WA1/2020 FRNT50**	**B.1.617.1 FRNT50**	**Fold Change**	**WA1/2020 FRNT50**	**B.1.617.2 FRNT50**	**Fold Change**
1	48	**F**	3/25/21	48	956	173	5.5	805	293	2.7
2	23	F	3/25/21	49	540	85	6.4	502	155	3.2
3	62	F	3/25/21	47	957	126	7.6	534	182	2.9
4	25	M	3/25/21	50	884	140	6.3	794	145	5.5
5	46	F	3/25/21	47	505	117	4.3	618	139	4.5
6	68	F	3/25/21	47	1074	100	10.7	907	187	4.9
7	24	F	3/25/21	35	3593	340	10.6	2091	601	3.5
8	63	F	3/26/21	50	1014	159	6.4	827	281	2.9
9	41	F	3/26/21	48	2322	175	13.3	1277	267	4.8
10	51	F	3/26/21	38	5330	826	6.5	3597	1460	2.5
11	40	F	3/26/21	50	759	118	6.4	958	371	2.6
12	66	F	3/26/21	51	1012	128	7.9	786	354	2.2
13	74	F	3/26/21	50	1411	186	7.6	1082	691	1.6
14	37	F	3/26/21	48	2007	372	5.4	1505	574	2.6
15	52	M	3/26/21	50	3159	595	5.3	2648	1364	1.9

Supplemental Table S6. FRNT50 results from Pfizer -BioNTech (BNT162b2) vaccinated samples

				Assay-1			Assay-2		
Sample	**Age Range**	**Sex**	**Days After Second Dose**	**WA1/2020 FRNT50**	**B.1.617.1 FRNT50**	**Fold Change**	**WA1/2020 FRNT50**	**B.1.617.2 FRNT50**	**Fold Change**
1	30 to 39	M	6	1237	261	4.7	903	437	2.1
2	40 to 49	M	7	937	148	6.3	647	270	2.4
3	30 to 39	M	27	560	82	6.8	430	227	1.9
4	30 to 39	F	8	1859	242	7.7	887	256	3.5
5	50 to 59	F	23	1812	327	5.5	1477	327	4.5
6	40 to 49	M	22	351	55	6.4	389	75	5.2
7	30 to 39	F	22	916	154	5.9	924	182	5.1
8	30 to 39	F	20	2106	103	20.4	578	173	3.3
9	30 to 39	M	9	2331	408	5.7	1178	422	2.8
10	60 to 69	F	21	1468	158	9.3	990	230	4.3

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